Addressing Mesocosm Data Requirements In The New EFSA Aquatic Guidance Document

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Cambridge Environmental Assessments

Introduction

- Broad overview of requirements for generating regulatory acceptable endpoints
  - ETO/ERO concepts
  - NOEC/LOEC determination
  - MDD values
  - Effect classes and assessment factors
- Overview of how we have attempted to address these requirements through appropriate experimental sampling
- Results
- Conclusions

Note: Exposure regimes not considered

Ecotoxicological Threshold Option

- ETO approach is reasonably straight forwards
- Necessary to identify sensitive taxonomic groups
  - Lower tier information
  - Read across information for substances with a similar MoA
  - Toxicity thresholds are generally comparable for organisms with short/simple and long/complex life cycles
  - Hence, assemblages are less important and can be based on sensitivity alone i.e. less important to include large univoltine taxa
- Requires ≥8 different populations of sensitive taxonomic groups to be present, for which statistical evaluation is possible (MDD)

Ecological Recovery Option

- ERO approach requires greater consideration of the assemblages present
  - Uni- Semi-voltine organisms
  - Organisms with a low dispersal rate
  - Macrophytes with relatively slow growth rates
- Otherwise higher uncertainty leads to higher AF values when extrapolating the NOEAEC to field populations
- Also recommend ≥8 different populations of sensitive taxonomic groups present, for which statistical evaluation is possible (MDD)
  - Should include ‘vulnerable taxa’

NOEC/LOEC Determination

- Statistically significant effects should be consistent on consecutive sampling occasions
  - Effects should be consistently direct or indirect
  - Effects to be considered in line with results from previous and subsequent sampling occasions
- Conclusions should not be solely based on statistical endpoints but also ecological factors
  - More important for recovery than for threshold concentrations
- As population level LOECs and NOECs can normally only be calculated for a few species, it is recommended that statistical analysis is performed on aggregated data

MDD values

- MDD values should be reported alongside NOEC values
- Values are aggregated into bands of classification:
  - Class 0 (>100%) No effects
  - Class I (90 to 100%) Large effects
  - Class II (70 to 90%) Large to medium
  - Class III (50 to 70%) Medium effects
  - Class IV (<50%) Small effects
- It is noted that MDD values for the majority of populations in mesocosm studies will be high due to a few organisms dominating the communities
- It is accepted that considering the variance in mesocosm studies Class I and Class II effects should be accepted

Effect Classes

- Effects are assigned to classes based on the magnitude and duration of effect:
  - Class 0 – Effects cannot be evaluated
  - Class 1 – No statistical or ecological effects
  - Class 2 – Slight effects
  - Class 3A/3B – Pronounced effects with recovery
  - Class 4 – Clear short term effects
  - Class 5A/5B – Pronounced long term effects with/without recovery
- Effect classes 1 and 2 may be used for the ETO option
- Effect class 3A should be used for the ERO option

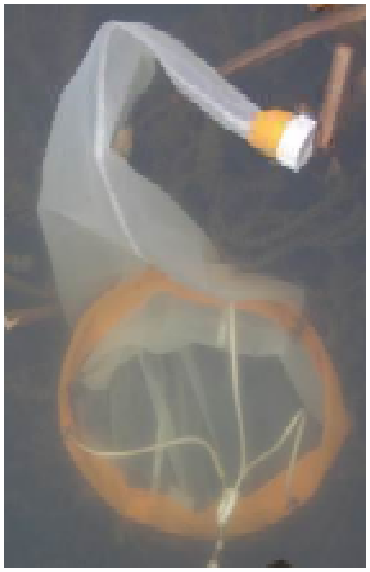
Assessment Factors

- Assessment factors should be based on the reliability of the endpoints
- Low assessment factors (2-3) recommended to be applied to effect classes 1 and 2 (ETO option)
- If an effect class 3A endpoint is used then higher assessment factors (3-4) should apply (ERO option)
  - The size of the AF should be sufficient to cover mesocosm to field extrapolation
  - The EFSA guidance goes into a lot of detail
- Decision trees are available to help determine Regulatory Acceptable Concentrations (RACs)

Practical Approaches

Insecticides

- General principle is to collect more robust data
- Sample as many niches as possible
- Benthos, Pelagic zone, Surface Dwellers, Resting organisms, insects
- *In-situ* assays for ‘vulnerable organisms’ of low density



Zooplankton MDDs

Day No.	Taxon/parameter							
	Lepadella sp.	Cyclopoida	Nauplii	Cladocera	Rotifera	Copepoda	Total number of Zooplankton	Total number of Zooplankton taxa
-8	51	71	15	65	38	15	23	42
-1	61	75	17	43	41	17	17	53
2	69	88	18	43	43	17	17	40
7	113	65	19	53	43	18	17	44
12	74	34	16	72	45	14	14	37
17	53	44	22	64	29	20	17	43
21	68	29	11	46	50	10	14	39
28	41	39	13	50	48	12	16	38
35	48	28	14	30	54	13	17	36
42	33	24	17	30	29	12	12	21
49	61	27	23	43	29	19	15	24
56	58	22	20	39	43	15	15	30
63	23	21	18	46	22	15	12	21
70	71	15	16	27	40	13	16	18

Note: MSD >100% = Class 0 (no effects can be determined), MSD 90-100% = Class 1 (only strong effects can be determined), MSD 70-90% = Class 2 (strong to medium effects can be determined), MSD 50-70% = Class 3 (medium effects can be determined), MSD 10-50% = Class 4 (low effects can be determined) (EFSA Aquatic Guidance Document, 2013)

Macroinvertebrate MDDs

Day No.	Taxon/parameter				
	Asellidae	Crangonyctidae	Group 4 (macrocrustacea)	Total number of macroinvertebrates	Total number of macroinvertebrate taxa
-17	25	34	24	19	43
-8	23	45	23	20	36
7	30	62	30	22	37
17	21	32	21	18	38
28	26	67	26	22	29
42	28	61	28	29	37
56	46	22	45	29	34
70	44	34	44	29	33
84	43	35	42	26	29
98	27	29	26	19	32
112	34	30	32	20	27

Note: MSD >100% = Class 0 (no effects can be determined), MSD 90-100% = Class 1 (only strong effects can be determined), MSD 70-90% = Class 2 (strong to medium effects can be determined), MSD 50-70% = Class 3 (medium effects can be determined), MSD 10-50% = Class 4 (low effects can be determined), (EFSA Aquatic Guidance Document, 2013)

Emergent Insect MDDs

Day No.	Taxon/parameter			
	Chaoboridae	Chironomidae	Total number of emergent insects	Total number of emergent taxa
-8	108	44	48	65
-1	81	56	50	63
7	87	77	62	54
17	61	34	62	58
28	47	36	60	46
35	82	56	75	81
42	61	67	60	37
49	81	42	67	51
56	72	33	45	50
63	74	31	48	41
70	106	60	50	63

Note: MSD >100% = Class 0 (no effects can be determined), MSD 90-100% = Class 1 (only strong effects can be determined), MSD 70-90% = Class 2 (strong to medium effects can be determined), MSD 50-70% = Class 3 (medium effects can be determined), MSD 10-50% = Class 4 (low effects can be determined), (EFSA Aquatic Guidance Document, 2013)

Insecticide Results - Overview

- Current testing approaches generally acceptable for zooplankton
  - MDD values <100% for many taxa
- More problematic for macroinvertebrates
  - Populations dominated by few taxa e.g. Asellus, Baetidae, Flatworms, Snails and Crangonyx
  - Highly variable for most other taxa
- Emergent insect data also variable
  - Use of totally enclosed mesocosms would increase numbers
  - Suitable for ETO endpoints
  - More challenging when looking at recovery

Herbicides

- Include as many taxa as is possible at densities that do not overload the system
- Quantitative and qualitative evaluation techniques
- Use of realistic community distributions
- Robust algae and periphyton sampling and counting methods

Phytoplankton MDDs

Day No.	Taxon/parameter							
	Anabaena sp.	Chroococcus sp.	Lyngbya sp.	Microcystis sp.	Oscillatoria sp.	Total Blue-green Algae	Total Blue green algae Taxa	Crypto monas sp.
-21	101	NC	50	38	NC	26	28	219
-7	178	NC	93	156	NC	80	74	538
1	82	177	282	219	NC	61	78	237
3	66	NC	57	15	423	23	39	424
7	102	267	60	48	NC	29	35	244
13	35	218	81	47	NC	20	52	151
16	81	109	201	85	NC	33	62	191
21	189	112	177	68	NC	41	56	780
28	67	83	306	47	161	38	50	176
35	101	67	67	32	250	27	34	329
42	22	63	21	173	98	19	30	152
49	60	136	44	18	166	19	45	323
56	75	40	30	65	182	22	26	339
63	15	123	48	69	145	12	36	200
70	80	117	31	73	241	24	47	244

Note: MSD >100% = Class 0 (no effects can be determined), MSD 90-100% = Class 1 (only strong effects can be determined), MSD 70-90% = Class 2 (strong to medium effects can be determined), MSD 50-70% = Class 3 (medium effects can be determined), MSD 10-50% =Class 4 (low effects can be determined), (EFSA Aquatic Guidance Document, 2013)

Macrophyte MDDs

Day No.	Taxon/parameter								
	Callitriche	Elodea	Filamentous	Glyceria	Hippuris	Myriophyllum	Potamogeton	Veronica	Total
-14	49	45	415	37	32	57	36	79	71
-1	42	64	148	47	81	46	44	35	43
7	46	47	153	40	96	48	55	23	37
21	51	70	88	55	79	47	42	29	20
28	50	42	140	57	55	38	50	39	23
42	72	47	229	54	65	33	31	30	25
56	161	35	244	51	126	59	46	31	23
70	349	39	202	83	134	42	45	25	26

Note: MSD >100% = Class 0 (no effects can be determined), MSD 90-100% = Class 1 (only strong effects can be determined), MSD 70-90% = Class 2 (strong to medium effects can be determined), MSD 50-70% = Class 3 (medium effects can be determined), MSD 10-50% = Class 4 (low effects can be determined), (EFSA Aquatic Guidance Document, 2013)

Herbicide Results - Overview

- Current testing approaches generally acceptable for algae, but…..
  - Cell densities in samples can be variable
  - Useful to count additional sub-samples until meaningful cell numbers are obtained for many taxa
- Macrophyte methods appear suitable for deriving endpoints with MDDs <100% for most taxa
  - Approaches are bespoke
  - Require greater standardisation between mesocosm facilities
- Functional parameters are sensitive and robust for detecting effects and functional recovery

Taxa	Nominal concentration				Relevant MSD class
	A	B	C	D	
Macroinvertebrates					
Asellidae	1	1	1	1	4
Group 1 (mayflies, stoneflies, diptera and moths)	1	1	1	1	1
Group 4 (macrocrustacea)	1	1	1	1	4
Group 5 (non-arthropod invertebrates)	1	1	1	1	3 to 4
Total No. macroinvertebrates	1	1	1	1	4
Total No. macroinvertebrate taxa	1	1	1	1	4
Macroinvertebrate community (PRC)	1	1	1	1	N/A
Sweep nets					
Notonectidae	1	1	1	1	1 to 2
Total No. macroinvertebrates	1	1	1	1	3
Total No. macroinvertebrate taxa	1	1	1	1	2 to 3
Emergent Insects					
Chaoboridae	3A	3A	3A	3A	2 to 3
Total Emergent Insects	1	1	1	1	3
Total Emergent Insect taxa	2	2	2	2	3
Emergent insect community (PRC)	3A	3A	3A	3B	N/A
Zooplankton					
Cyclopoida	1	1	1	1	4
Daphnia sp.	1	1	1	1	1
Lecane sp.	1	1	1	1	1
Lepadella sp.	1	1	1	1	2 to 4
Nauplia	1	1	1	1	4
Total Cladocera	1	1	1	1	3 to 4
Total Rotifera	1	1	1	1	4
Total Copepoda	1	1	1	1	4
Zooplankton community (PRC)	1	1	1	1	N/A
Overall NOEC	<1µg a.s./L)				
Overall NOEAEC (Class 3A)			X		
Overall NOEAEC (Class 3B)				X	

Overall results

Conclusions

- The new guidance increases requirements for considerations in the design and conduct of mesocosm studies
- These requirements have generally been achieved in historical studies
- Some work may be needed to provide data on additional species
- Some recent developments have addressed the new requirements to ensure these are met for herbicide studies in particular